J. Anat. (1971), **110**, 1, pp. 73–79
With 8 figures
Printed in Great Britain

Histochemical study of ubiquinones in the central nervous system of mice

F. HERNÁNDEZ, J. R. IGLESIAS AND J. MARTÍNEZ DE MORENTIN

Department of Histology (CIB 'Félix Huarte'), University of Navarra, Pamplona, Spain

(*Accepted* 3 *June* 1971)

INTRODUCTION

The quinones are important in biological systems; they are easily reduced to quinoles, and these in turn readily oxidized to quinones. The quinones made their first impact on biochemistry when vitamin K was discovered nearly 30 years ago, and a new type of quinone, ubiquinone or coenzyme Q, has recently provided important information relative to some fundamental problems in biology. It was discovered by Morton and others while studying the liposoluble vitamins (Morton, 1961) and also by Crane, Hatefi, Lester & Widmer (1957) while studying the respiratory chain. Green & Brierley (1965) considered that ubiquinone functions in the respiratory chain as a redox mechanism transporting electrons between the reduced flavoproteins and the cytochromes.

Biochemical extraction and chromatography were the only methods used for localizing ubiquinone prior to the histochemical method of Tranzer & Pearse (1963), which was applicable to ubiquinone and other similar compounds. This method consisted in using hydroquinone as an electron donor in conjunction with a tetrazolium salt in the following manner:

$$\text{hydroquinone} \xrightarrow{2e} \text{ubiquinone} \xrightarrow{2e} \text{tetrazolium} \xrightarrow{2e} \text{formazan.}$$

By means of biochemical methods, Green (1961) confirmed a high concentration of ubiquinone in the rat's cerebrum and no detectable concentration in the serum. We therefore performed this investigation to ascertain the histological distribution of this substance within the central nervous system.

MATERIAL AND METHODS

After decapitation, the brain and spinal cord were removed from male mice 4–6 months old, frozen in liquid nitrogen at -170 °C and stored at -70 °C. They were frontally oriented in the cryostat, and 8 μm serial sections were obtained. Ubiquinone staining, following the method of Tranzer & Pearse, was carried out in every seventh section.

The technique was as follows:

(1) The unfixed sections were allowed to dry for 30 min at room temperature.

(2) These sections were incubated at 37 °C for 40 min in a solution of 0·25 ml of

3-(4,5-dimethyl-thiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT tetrazolium, Sigma Chemical Company, St Louis, Mo.), 1 mg/ml in distilled water; 0·05 ml of 0·5 M cobalt chloride; 0·50 ml of 0·2 M tris buffer, pH 7·4; 2 mg of catalase; and 0·10 ml of hydroquinone, 40 mg/ml in tris buffer, pH 7·4. This solution must be absolutely fresh, and filtered before use.

(3) The reaction was stopped and the sections were fixed for 15 min in 10 % formalin at room temperature, followed by a brief washing under running tap water and subsequent mounting in glycerol jelly.

In all, 15 mice were studied. Positivity was classified from 1 to 4 according to the intensity of the formazan precipitate as observed in the light microscope. As a subjective reference standard, we used the positivity of the molecular layer of the cerebellum, which we classified constantly as an intensity of 2.

Control sections received identical treatment, with the exception that the hydro-

Table 1

Figure(s)	Location	Area and/or element	Positivity
	White matter	—	0
	Grey matter	—	1–2
	Ependyma	—	1–2
1	Choroid plexus	—	4
2	Sympathetic ganglia	Large neurons	3
		Other neurons	1–2
3	Spinal ganglia	Large neurons	4
		Other neurons	2–3
5	Spinal cord	Substantia gelatinosa; periependymal region; dorsal horn; various isolated cells, principally in ventral horn	3–4
		Remaining grey matter	2
6	Medulla oblongata	Various isolated cells (considered to be reticular)	4
		Outer part of trigeminal spinal nucleus, area postrema, arcuate and olivary nuclei	3
		Dorsal nucleus of vagus, nucleus ambiguus, hypoglossal nucleus, nucleus of tractus solitarius, lateral reticular nucleus	3–4
	Pons	Cells of white and grey reticular substance and extrapyramidal neurons of the cerebellar peduncles	4
		Vestibular, pontine, and olivary nuclei	3
4, 6	Cerebellum	Molecular layer and Purkinje cells	2
		Granular layer	3–4
		Nuclei	3–4
7, 8	Cerebrum	Cortex in general, central grey matter	2
		Subcommissural organ	4
		Epithelium of pineal recess	3
		Isolated cells in thalamic nuclei	2–3
7	Pineal	Uniform positivity throughout	3
	Hypophysis	Anterior lobe	2
		Posterior lobe	1
		Pars intermedia	3

quinone solution in the first incubating medium was replaced by an equal volume of distilled water (Tranzer & Pearse, 1965). In some controls, acetone (99·5 Merck) was added to the preparations and washed out by progressive dilutions from alcohols to water before incubation.

RESULTS

The intensity of any given positive zone was identical in all mice studied (see Table 1). The characteristic formazan precipitate was never observed in control sections incubated with MTT tetrazolium in the absence of hydroquinone.

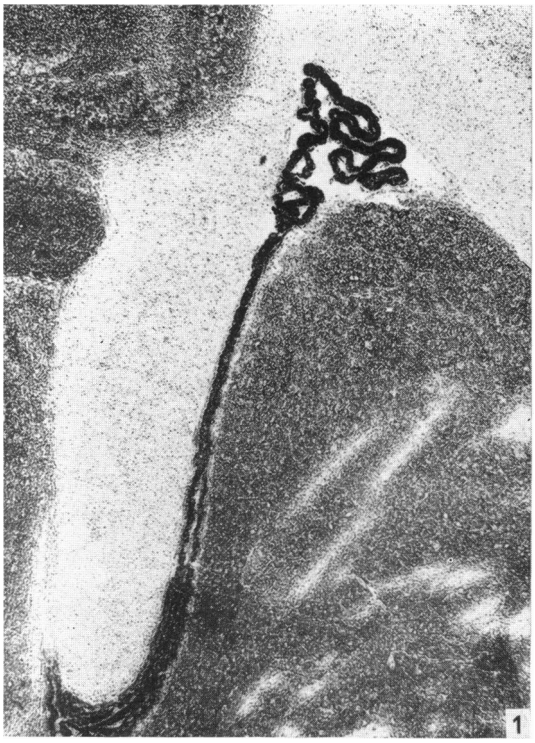
DISCUSSION

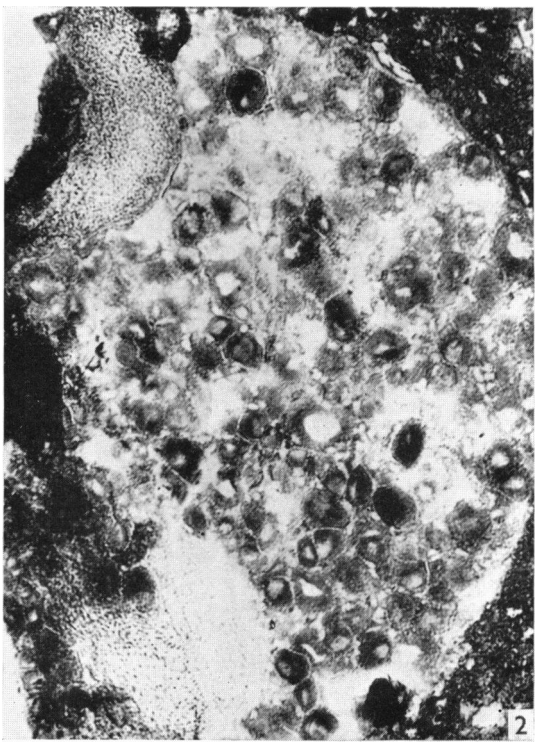
In this histochemical reaction the ubiquinone receives electrons from hydroquinone and transfers electrons directly to the tetrazolium salt, thus constituting a ubiquinone-tetrazolium reductase system. Ubiquinone possibly belongs to the mitochondrial electron and microsomal electron transport system (Glenner, 1965). Hess & Pearse (1963) have reported the presence of a $NADH_2$-quinone (menadione)-tetrazolium reductase in both mitochondrial and soluble brain fractions. However, Carmichael (1963) noted that phospholipids might cause false localization of diffusible quinone in view of the fact that choline and other phospholipid bases bind hydroquinone by salt linkage. Carmichael & Mander (1967) have stained amino groups by means of formazan in sections incubated in a mixture of hydroquinone and 3-(4,5-dimethyl thiazolyl-2)-2,5-diphenyl-2H-tetrazolium bromide at pH 6·0 and have therefore expressed doubt about the validity of Tranzer & Pearse's method (1963) for the staining of ubiquinone.

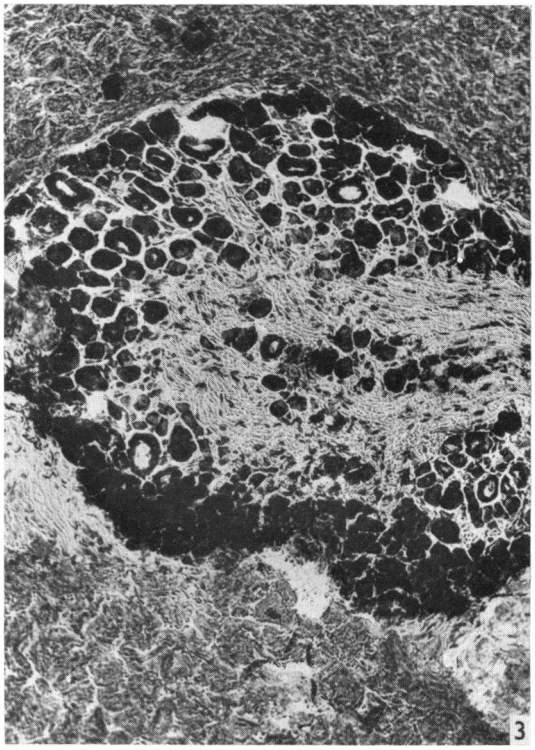
Different results were also obtained in our investigation upon changing the pH or the time of incubation, or suppressing the catalase, or adding acetone or propanol before incubation. But the results set out above were obtained by following strictly the method of Tranzer & Pearse (1963). The rationale of this method was strengthened by Horwitz, Benitez & Bray (1967), who investigated the role played by ubiquinone in the succinic–tetrazolium–reductase reaction. Fresh cryostat sections of normal rat material were treated with acetone to remove ubiquinone. Using sodium succinate as a substrate, they found that the sections which had had the ubiquinone extracted did not reduce the tetrazolium salt to formazan; the reduction reaction did occur in controls, which had first been treated with acetone to remove the ubiquinone, and then subsequently had added to them the ubiquinone–lecithin complex.

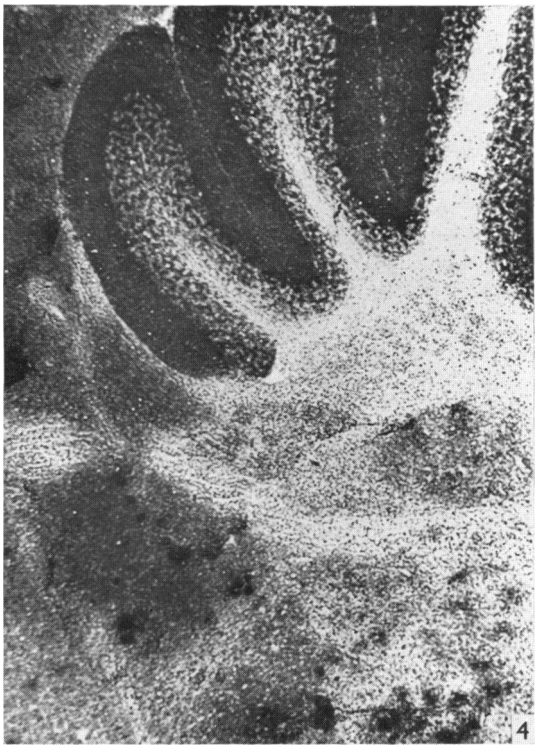
We observed a strong positivity, in the spinal cord as well as in the medulla oblongata, in those cells perhaps involved in thermal and pain perception of a protopathic type (substantia gelatinosa and trigeminal spinal nucleus); there was also a strong reaction in the spinal ganglia. A high positivity also existed in the association neurons and in particular those related to the extrapyramidal motor system; the cortico-ponto-cerebellar-olivo-rubro-spinal system was well delineated in the spinal cord, medulla oblongata, pons, cerebellar peduncles, vestibular nuclei, and central cerebellar nuclei.

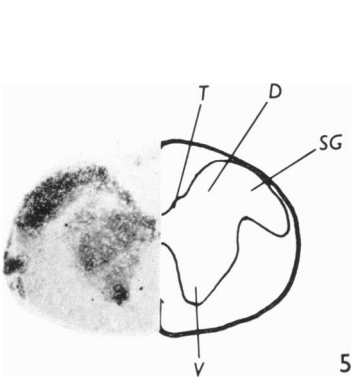
In general, those cells with thick axons (presumably of high conduction velocity) are highly positive. This perhaps could be attributed to a greater activity in the electron transport chain. The difference between the white and grey matter suggests

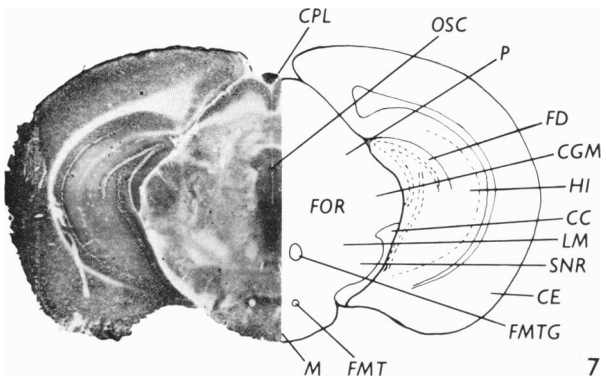

1

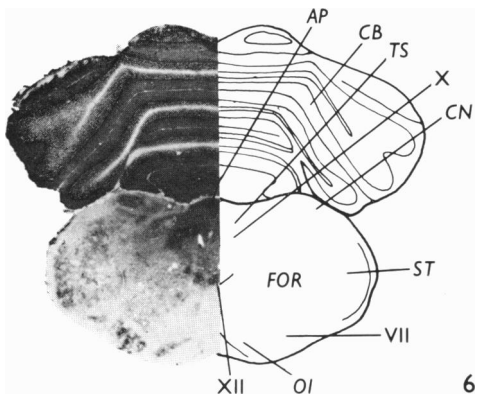

2

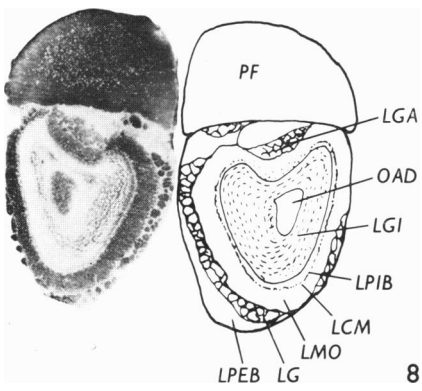

3


4









Figs. 5–8. Distribution of ubiquinone.

Fig. 5. In spinal cord. *D*, dorsal horn; *V*, ventral horn; *T*, thoracic nucleus; *SG*, substantia gelatinosa.

Fig. 6. In medulla and cerebellum. *AP*, area postrema; *CB*, cerebellum; *FOR*, reticular formation; *OI*, olive; *CN*, nucleus cuneatus; VII, nucleus of VII; X, nucleus of X; XII, nucleus of XII; *ST*, nucleus of spinal tract of V; *TS*, nucleus of tractus solitarius.

Fig. 7. In cerebrum. *CC*, crus cerebri; *CE*, entorhinal cortex; *CGM*, medial geniculate body; *CPL*, pineal body; *FD*, dentate fascia; *FMT*, mamillothalamic tract; *FMTG*, mamillotegmental tract; *FOR*, reticular formation; *HI*, hippocampus; *LM*, medial lemniscus; *OSC*, subcommissural organ; *SNR*, substantia nigra; *M*, mamillary complex; *P*, putamen.

Fig. 8. In olfactory bulb. *LCM*, mitral layer; *LG*, glomerular layer; *LGA*, glomerular layer of accessory bulb; *LGI*, internal granular layer; *LMO* molecular layer; *LPEB*, external plexiform layer; *LPIB*, internal plexiform layer; *PF*, frontal pole; *OAD*, dorsal part of anterior olfactory nucleus.

Fig. 1. Pronounced positivity of choroid plexus (lateral ventricle). × 40.

Fig. 2. Paravertebral sympathetic ganglion showing differing positivities in its cells. × 100.

Fig. 3. Spinal ganglion showing great positivity of most of its neurons. × 100.

Fig. 4. Strongly positive cells in the cerebellar cortex, inferior cerebellar peduncles, and cerebellar nuclei. Sagittal section. × 40.

that oxidative phosphorylation in the white matter is less intense than in the grey. The positivity of neuropil may be due to neuronal expansions and to glia, although, according to Friede (1966), the glia normally has little oxidative enzyme activity. There are differences in ubiquinone activity between the glia of the grey matter and the white matter; the latter being practically negative. According to Hyden (1967), this may be due to the neurone–glia relationship being the last one influenced metabolically by the active perikaryon of the neurons.

It is interesting that our findings coincide almost entirely with Friede's (1961) histochemical map of oxidative enzymes. They coincide especially with the distribution of NADH diaphorase (Friede & Fleming, 1962) in the human brain; NADH diaphorase, like ubiquinone, acts in the oxidation–reduction system and is mitochondrial as well as extramitochondrial.

SUMMARY

The histochemical distribution of ubiquinone at different levels of the central nervous system, as well as in the spinal and sympathetic ganglia, hypophysis and pineal body, was observed.

Strong activity was detected in the choroid plexus, in the majority of the neurons of the spinal ganglia, in the substantia gelatinosa, in isolated cells of the reticular substance within the medulla oblongata and pons, in the nuclei of the cerebellum and in the subcommissural organ. Moderate activity was observed in some of the neurons of the sympathetic ganglia, in the periependymal region of the spinal cord, within some isolated cells of the anterior horn, in the area postrema, the pontine and vestibular nuclei, the cerebellar granular layer, and in the pineal gland and the pars intermedia of the hypophysis. The remainder of the grey substance had less positivity, and no reaction was observed in the white substance of the central nervous system.

The technical assistance of Miss Reyes Esparza and Miss Pilar Ibarra is greatly appreciated.

REFERENCES

CARMICHAEL, G. G. (1963). A tetrazolium salt reduction method for demonstrating lipoproteins in tissue sections. *J. Histochem. Cytochem.* **11**, 738–740.

CARMICHAEL, G. G. & MANDER, S. T. K. (1967). *p*-Hydroquinone and *p*-benzoquinone as histochemical reagents. I. A new tetrazolium method for amino groups, and the mechanism of formazan production of paraffin and fresh frozen sections. *J. Histochem. Cytochem.* **15**, 404–408.

CRANE, F. L., HATEFI, Y., LESTER, R. L. & WIDMER, C. (1957). Isolation of a quinone, from beef heart mitochondria. *Biochim. biophys. Acta* **25**, 220–221.

FRIEDE, R. L. (1961). In *A Histochemical Atlas of Tissue Oxidation in the Brain Stem of the Cat*. New York: Karger.

FRIEDE, R. L. (1966). In *Topographic Brain Chemistry*. New York: Academic Press.

FRIEDE, R. L. & FLEMING, L. M. (1962). A mapping of oxidative enzymes in the human brain. *J. Neurochem.* **9**, 179–198.

GLENNER, G. G. (1965). In *Neurohistochemistry*, (ed. C. W. M. Adams). Amsterdam: Elsevier.

GREEN, D. E. & BRIERLEY, G. P. (1965). In *Biochemistry of Quinones*, (ed. R. A. Morton). London: Academic Press.

GREEN, J. (1961). In *Ciba Foundation Symposium on Quinones in Electron Transport*, (ed. G. E. W. Wolstenholme and C. M. O'Connor). London: Churchill.

HESS, R. & PEARSE, A. G. E. (1963). Pathways of reduced pyridine nucleotide oxidation in rat brain homogenate demonstrated by a tetrazolium method. *Biochim. biophys. Acta* **71**, 285–294.

HORWITZ, C. A., BENITEZ, L. & BRAY, M. (1967). The effect of coenzyme 2 on the histochemical succinic tetrazolium reductase reaction: a histochemical study. *J. Histochem. Cytochem.* **15**, 216–224.

HYDEN, H. (1967). In *The Neuron.* Amsterdam: Elsevier.

MORTON, R. A. (1961). In *Ciba Foundation Symposium on Quinones in Electron Transport*, (ed. G. E. W. Wolstenholme and C. M. O'Connor). London: Churchill.

TRANZER, J. & PEARSE, A. G. E. (1963) Cytochemical demonstration of ubiquinone in animal tissues. *Nature, Lond.* **199**, 1063–1066.

TRANZER, J. P. & PEARSE, A. G. E. (1965). The electron cytochemistry of ubiquinone in mammalian heart, demonstrated with a monotetrazolium salt. *Histochemie*, **4**, 502–506.